

*IN THE SPECIFICATION:*

Please make the following amendments to the specification:

(~~strikethrough~~ indicates deletions, underline indicates insertions & *italics* indicates underline)

Page 6, delete the paragraph beginning at line 15.

Page 6, the paragraph beginning at line 18:

~~Figure 5~~ 4 shows sites at which the genome is cut by the restriction enzyme EcoRI and construction of recombinant plasmids carrying HTLV-III DNA.

Page 6, the paragraph beginning at line 21:

~~Figure 6 is an immunoblot showing the positions on nitrocellulose blots of peptides produced by bacterial cells transformed by recombinant constructs ompA1-R-6, ompA2-R-7 and ompA3-R-3, into which a 1.1Kb EcoRI HTLV-III cDNA restriction fragment had been inserted.~~ Figure 6a ~~5~~ shows the nucleotide sequence of the ompA signal peptide and the pertinent region of recombinant plasmids ompA1-R-6; ompA2-R-7 and ompA3-R-3.

Page 6, delete the paragraph beginning at line 30 and continuing through page 7, line 3.

Page 7, delete the paragraph beginning at line 4.

Page 7, the paragraph beginning at line 12:

~~Figure 9~~ 6 represents the open reading frame expression vector pMRIOO having HTLV-III DNA.

Page 7, delete the paragraph beginning at line 14.

Page 15, the paragraph beginning at line 24:

The cells from the selected colonies are grown in culture. The culture is spun down and the cell pellet broken. Total cellular protein is analysed by being run on an SDS polyacrylamide gel. The fusion proteins are identified at a position on the gel which contains no other protein. (~~Figure 4~~)

Page 28, the paragraph beginning at line 5:

Several gene segments that encode peptides showing immunoreactivity with anti-HTLV-III antibodies were demonstrated. Among these is a 1.1 Kb EcoRI restriction fragment. This fragment was inserted into ompA vectors in all three reading frames (Figure 5 4). Cells were grown at 37°C in L broth containing 100mg/ml. ampicillin to an <sup>OD</sup>600 of 0.2. At this time, the cell cultures were divided into two aliquots. IPTG was added to one aliquot to a final concentration of 2mM (induced). IPTG was not added to the other aliquot (uninduced). Upon IPTG induction, transformants of all three plasmid constructs (designated OmpA<sub>1</sub>-R-6 (O1R6), OmpA<sub>2</sub>-R-7 (O2R7), and OmpA<sub>3</sub>-R-3 (O3R3)) produced a 15 Kd peptide that is strongly reactive with anti-HTLV-III antibodies in sera from AIDS patients (Figure 6 lane 1, purified HTLV-III virions; lanes 2 and 3, O1R6 uninduced and induced; lanes 4 and 5, O2R7 uninduced and induced; lanes 6 and 7 O3R3 uninduced and induced). This reactivity is not detected when sera from normal individuals is used.

Page 28, the paragraph beginning at line 26:

DNA sequence data of the HTLV-III genome indicates that there is an open reading frame inside the *pol* gene located at the 5'-end of the EcoRI fragment. DNA sequence analysis of the three recombinant constructs, O1R6, O2R7 and P3R3, confirmed that each of these recombinants has a different reading frame of the HTLV-III plus strand coupled to the coding sequence of each vector. Only in O3R3 is the reading frame of the inserted DNA in phase with that set by the signal peptide in the ompA vector; in O1R6 and O2R7 the *pol* gene segment DNA is out of phase (Figure 6a 5).

Page 29, the paragraph beginning at line 17:

In addition to the 15 Kd peptide, the O3R3 construct, in which the reading frame of the HTLV-III DNA *pol* gene is in phase with that set by the vector, produced two additional peptides about 19 Kd and 16.5 Kd in size (Figure 6). It is possible that the 19 Kd peptide contains an additional 35 amino acid residues, 21 of which are from the signal peptide encoded by the ompA<sub>3</sub> vector and 14 encoded by the inserted HTLV-III DNA itself. The 16.5 Kd peptide may be the processed 19 Kd peptide in which the signal peptide is cleaved.

Page 29, the paragraph beginning at line 28:

The O1R6 and O2R7 constructs also produces another peptide of about 17.5 Kd (Figure 6) and weakly reactive with sera of AIDS patients. The origin of the peptide is not clear. The 1.1 Kb EcoRI fragment contains a second potential coding region designated as

the short open reading frame (SOR) extending from nucleotide position 360 to 965 (Figure 5 4). Four of the five AUG methionine codons in this region are near the 5'-end of this open reading frame. This DNA segment could encode peptides of 192, 185, 177 or 164 amino acid residues. However, there is no clearly recognizable ribosome binding site at the 5'-end of this open reading frame.

Page 30, the paragraph beginning at line 9:

Further evidence also supports the conclusion that the 15 Kd peptide is indeed derived from the *pol* gene. First, deletion of the 3'-end StuI to EcoRI fragment from the 1.1 Kb EcoRI insert from O1R6, O2R7 and O3R8 (Figure 5 4) does not affect the synthesis of the 15 Kd peptide. Second, clones containing only the 5'-end EcoRI to NdeI fragment still produce the same 16 Kd peptide. Finally, several recombinant clones containing various DNA fragments having the SOR coding sequence properly inserted into the open reading frame cloning vector, pMR100, produced lambdaCI-HTLV-III B-galactosidase tripartite fusion proteins which have very little immunoreactivity with anti-HTLV-III antibodies present in sera from AIDS patients.

Page 30, the paragraph beginning at line 24:

Significant immunoreactivity against the 15 Kd peptide derived from the viral *pol* gene in sera from AIDS patients was detected. The identity of this immunoreactive peptide, with respect to the banding pattern of HTLV-III virion antigen in SDS-polyacrylamide gel electrophoresis, was determined by means of a competition inhibition immunoassay. Purified HTLV-III virions were treated with SDS, electrophoresed, and electroblotted onto a nitrocellulose filter. Identical filter strips containing disrupted HTLV-III virions were incubated with well characterized serum from an AIDS patient in the presence or absence of lysates of O1R6, O2R7, or control bacterial clones. The specific immunoreaction between anti-HTLV-III antibodies present in sera of the AIDS patients and the blotted virion proteins were then revealed by <sup>125</sup>I-labeled goat anti-human antibody. As shown in Figure 7, lysates Lysates of O1R6 block the immunoreactivity of the viral p31 protein with the AIDS serum, while lysates of control cells do not. This result suggests that the recombinant 16 Kd peptide encoded by 3'-end of the viral *pol* gene is also a part of another virion protein, p31, in contrast to the view shared by some that p31 is a cellular protein which co-purifies with HTLV-III virions.

Page 31, the paragraph beginning at line 17:

The prevalence in the sera of AIDS patients of antibodies against the 15 Kd peptide was also evaluated. In Western blot analysis employing the lysate of O1R6 as the source of antigen, a panel of coded sera from AIDS patients and normal healthy individuals was tested. All of the 20 AIDS sera and none of the 8 normal controls reacted with the 15 Kd peptide. Representative results are shown in (Figure 8). These data indicate that most, if not all, AIDS patients produce antibodies against the viral p31 protein.

Page 32, the paragraph beginning at line 4:

HTLV-III DNA was excised from lambda BH-10, which is a previously constructed recombinant lambda phage containing a 9 Kb segment of HTLV-III DNA inserted into the vector lambdagtwes lambda B (Figure 2a). This HTLV-III DNA was sonicated and DNA fragments of about 0.5 Kb purified by gel electrophoresis, end repaired, and inserted into the SmaI site of the open reading frame (ORF) vector, pMR100 (Figure 9 6). This vector contains a bacterial *lac* promoter DNA segment linked to a second DNA fragment containing a hybrid coding sequence in which the N-terminus (5' segment) of the lambda CI gene of bacteriophage lambda is fused to an N-terminal-deleted *lacIZ* gene (3' segment). A short linker DNA fragment, containing a SmaI cloning site, has been inserted between these two fragments in such a manner that a frame shift mutation has been introduced upstream of the *lacIZ*-coding DNA. As a result, pMR100 does not produce any detectable B-galactosidase activity when introduced into cells of the Lac<sup>-</sup> host *E. coli* LG90. The insertion of foreign DNA containing an open reading frame, in this case the HTLV-III DNA, at the SmaI cloning site can reverse the frame shift mutation if the inserted coding sequence is in the correct reading frame with respect to both the lambdaCI leader and the *lacIZ* gene. Transformants were screened on MacConkey plates to detect individual clones that expressed B-galactosidase enzymatic activity *in situ*.

Page 33, the paragraph beginning at line 14:

The proteins produced by the Lac<sup>+</sup> clones were analyzed by resolving cell lysates on 7.5% SDS-polyacrylamide gels along with those of the control Lac<sup>+</sup> clone pMR200, which produced a lambdaCI-B-galactosidase fusion protein. The *lacIZ* gene in pMR200 is identical to that in pMR100 except that it has a single base pair deletion which brings it in phase with the lambdaCI gene to produce an active B-galactosidase. By virtue of the very large size of the B-galactosidase and its fusion protecins, they are separated from the bulk of proteins in the cell lysates on the SDS-polyacrylamide gels and can be easily identified by Coomassie brilliant blue staining as shown in Figure 10a. Some of the Lac<sup>+</sup> clones containing HTLV-III

DNA produce polypeptides that are larger (15,000 to 27,000 daltons) than the lambdaCI-*lacIZ* fusion protein. These findings are consistent with data that the DNA inserts are up to 700 bp long. The B-galactosidase fusion proteins accounted for about 1-2% of total cellular protein.

Page 34, the paragraph beginning at line 1:

The peptides produced by the Lac<sup>+</sup> clones were examined by Western blot analysis for immunoreactivity with sera from AIDS patients. After the lysates of Lac<sup>+</sup> clones were electrophoresed in SDS-polyacrylamide gels, they were electro-transferred to nitrocellulose filters. These protein blots were first reacted with AIDS patient sera and then with <sup>125</sup>I-labeled goat anti-human IgG. ~~The autoradiograph in Figure 10b shows the immunoreactivity of a representative fused protein with the serum from an AIDS patient.~~ The recombinant peptides also reacted with anti-B-galactosidase antiserum, consistent with the proposition that they had the general structure lambdaCI-HTLV-III peptide-LacIZ. From the immunoreactivity pattern of the negative controls, pMR100 and pMR200, which do not contain an HTLV-III DNA insert, it is evident that this particular AIDS serum contains antibodies reactive with several bacterial proteins of the host *E. coli*. This is not surprising, since AIDS patients are usually infected with a number of bacteria. Absorbing AIDS patient sera with Sepharose 4B conjugated with *E. coli* extract reduced the background immunoreactivity to some extent but did not completely eliminate it.